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EFFECTS OF METOLACHLOR AND ALACHLOR ON PERMEABILITY AND LIPID SYNTHESIS IN SOME PLANTS

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ABSTRACT

This study was carried out to investigate the effects of metolachlor on root permeability and to determine whether metolachlor or alachlor inhibit plant lipid synthesis.

Metolachlor at 30 and 40 ppm increased the leakage of previously absorbed ^{32}P-labelled orthophosphate from the roots of onion (a susceptible species) by 14 and 41 times the control values respectively. A significant amount of ^{32}P leaked from the roots of the moderately susceptible species (wheat and tomato) whereas no significant loss of ^{32}P occurred to two tolerant species (*Pisum* and corn). At 10 or 20 ppm, 1,8-naphthalic anhydride prevented ^{32}P leakage from onion roots in the presence of 30 ppm metolachlor. High concentrations of naphthalic anhydride, however, promoted ^{32}P leakage and did not protect onion roots from the leakage induced by high concentrations (40 ppm) of metolachlor. Neither metolachlor nor alachlor at 40 ppm, inhibited the uptake of acetate-2-^{14}C or malonic acid-2-^{14}C into excised wheat root tips.

Incorporation of ^{14}C-choline chloride into phosphatidylcholine was not significantly inhibited by metolachlor.

KEY WORDS: Plant physiology, metolachlor, alachlor, radioactive labeling.

INTRODUCTION

Metolachlor is a selective herbicide used for the control of grass weeds, nutsedge and broadleaf species in corn. Other crops showing tolerance include soybean, peanut, potato and certain vegetables.

Numerous studies have been conducted to determine the mechanism of action of α-chloroacetamides. Studies of photosynthesis, respiration, α-amylase

synthesis, RNA synthesis, protein synthesis and lipid synthesis have failed, however, to elucidate the primary mechanism of action (Chandler et al. 1972; Devlin & Cunningham 1970; Diner et al. 1978; Duke et al. 1975; Jaworski 1956; Moreland et al. 1969; Sasaki et al. 1966 and Truelove & Diner 1978).

There is a considerable literature on the effects of metolachlor on the morphology of higher plants.

Dixon (1981), came to the conclusion that 100 ppm of metolachlor reduced shoot growth of maize by 65%, and the uptake of the herbicide was twice in maize as in nutsedge.

Shoot and root growth are inhibited by α-chloroacetamides (Deal & Hess 1980; Duke et al. 1975; Keeley et al. 1972 and Pillai et al. 1979). Deal & Hess (1980), found that growth of *Pisum sativum* and *Avena sativa* were inhibited by both metolachlor and alachlor. They suggested that growth inhibition by these herbicides resulted from an inhibition of both cell division and cell enlargement.

Protein synthesis is inhibited by the effect of certain α-chloroacetamides (Mann et al. 1965). Pillai et al. (1979), found that, with metolachlor, leucine incorporation into protein was inhibited only at concentrations of 1×10^{-4}M and higher.

Diner et al. (1978), found that α-amylase synthesis was inhibited by alachlor and metolachlor only at concentrations in excess of 1×10^{-3}M.

While conducting studies on the effects of α-chloroacetamides on root growth, the nutrient solution containing 40 ppm metolachlor (where the onion roots were growing) became turbid after 48 hrs and extensive colonies of fungi and bacteria were associated with the roots. This observation suggested the possibility that the herbicide was stimulating the growth of these organisms by causing the leakage of substance from the roots that stimulated microbial growth. This further suggested that the herbicide may be causing a loss of root cell membrane integrity.

Studies of the interaction between herbicide and lipids (one of the two major components of cell membrane) have been carried out by Mann & Pu (1968). Using excised hypocotyls of Hemp sesbania, they demonstrated that a number of herbicides, including the α-chloroacetamide can inhibit lipoxygenases as measured by a reduction in the incorporation of malonic acid-2-^{14}C into lipid.

MATERIALS AND METHODS

Seeds of *Allium cepa* L. purchased from The Ministry of Agriculture, Cairo, were surface sterilized, and their basal ends were submerged in beakers containing half-strength nutrient solution (Hoagland & Arnon 1950). Roots were allowed to grow for 10 days at 27°C and constant light intensity of about (6,000 lux) in a growth chamber. Nine onions with a similar number of roots

were transferred to beakers containing 60 ml of phosphorus deficient, half strength nutrient solution containing 5 μCi ^{32}P as orthophosphate (sp. act. 0.8 mCi/ml). The bulbs were placed so that only the roots were in contact with the radioactive solution. After 24 hrs, onions were removed from the ^{32}P solution and the roots were washed three times with fresh nutrient solution before transferring them to beakers containing 60 ml of half strength nutrient solution, which contained 100 μg/ml penicillin and 40 μg/ml chloramphenicol to inhibit microbial growth. A stock solution of metolachlor in ethanol was added to give final concentrations of 0, 30 or 40 ppm herbicide in each of three replications. A similar amount of ethanol was present in the no-herbicide controls. Duplicate 0.2 ml samples of the solutions were withdrawn after 0, 4, 8 and 12 hrs and then every 12 hrs for 6 days. These samples were radio assayed by Packard liquid scintillation, type spectrometer series 4000.

Similar studies were conducted using roots of *Lycopersicon lycopersicum* L., *Zea mays* L., *Triticum aestivum* L. and *Pisum sativum* L.

To show the effect of naphthalic anhydride on metolachlor-induced ^{32}P leakage from onion roots, the experiments were conducted as described above, but naphthalic anhydride was added to some of the treatments. Preliminary experiments were carried out using aqueous naphthalic anhydride suspensions containing 0.1 and 0.4% respectively, applied both alone and with 40 ppm metolachlor. Later experiments used lower rates of both 10, 20 and 30 ppm metolachlor.

Wheat grains were germinated for 72 hrs in the dark at 28°C on filter paper moistened with 1 x 10^{-3}M $CaCl_2$. After harvesting, 1 cm long root tips were excised and held in cold aqueous sucrose until a sufficient number had been collected. Groups of 100 root tips were removed from the sucrose and transferred to flasks containing 2 ml of 0.01 M potassium phosphate, 1% sucrose and 10 μg/ml chloramphenicol. Herbicide, dissolved in ethanol, was added to give a final herbicide concentration of 40 ppm. Ethanol was present in all treatments at a concentration of 0.5%, 5 microcuries of malonic acid-2-^{14}C (sp. act. 50.4 mCi/ml) were added as a precursor for lipid synthesis.

Flasks were covered with black polyurethane and the root tips were incubated for 6 hours at 30°C in a shaking water bath. Following incubation, root tips were removed, washed twice with non labeled malonic-acid (200 μg/ml) and twice with water. The root tips were then frozen at 0°C, freeze dried and weighed before grinding in a mortar. The tissue was transferred into 20 ml of chloroform:methanol (2:1) mechanically stirred for one hour and filtered through a double thickness Whatman No. 1 filter paper.

The filtrate was dried under a stream of N_2. The crude lipid extract was dissolved in 5 ml chloroform, 2 ml H_20 was added, and the mixture was shaken. The lower chloroform layer was removed and passed through a small glass column containing anhydrous sodium sulfate to remove residual water

and water soluble non lipid residues. This fraction and the upper, aqueous layer (non incorporated precursor) were both sampled and radio assayed by liquid scintillation.

Further studies were conducted in the same manner, but using acetate-2-^{14}C (sp. act. 59.2 mCi/ml) as lipid precursor instead of malonic acid-2-^{14}C. Similar studies were conducted using a longer incubation period of nine hours. Lipid extracts were spotted on pre-coated TLC plates and separated using petroleum ether:diethyl ether:acetic acid (79:10:1).

Radioactive areas were detected by scanning the plate with a TLC radioscanner. The phospholipids, which remained on the TLC plates, were extracted from the silica gel with chloroform:methanol:water (5:5:1). Extracts were dried under N_2 and redissolved in chloroform prior to further separation by TLC. The remaining lipid classes on the TLC plates were removed by scraping the radioactive areas of silica gel into vials containing scintillation fluid. The vials were then assayed by liquid scintillation.

To study the incorporation of choline chloride into phosphatidylcholine, the experiments were carried out in a similar way to malonic acid-2-^{14}C and acetate-2-^{14}C incorporation studies with the following exceptions 1.0 μCi choline chloride-1-2-^{14}C (sp. act. 5.4 mCi/ml) was used as a precursor to phosphatidylcholine and the preliminary TLC of the lipid extract to separate the neutral lipids from the polar phospholipids was omitted. Radioactivity remaining at the origin of the TLC plate after separation by polar solvent system was eluted, concentrated and identified as choline chloride using paper chromatography and a solvent system of n-butanol:ethanol:acetic acid:water (8:2:1:3).

RESULTS AND DISCUSSION

Preliminary studies with onion had suggested that metolachlor was affecting the permeability of root membranes, causing leakage of plant metabolites.

To determine whether the effects of metolachlor on permeability were related to the phytotoxicity range of the herbicide, several species differing in sensitivity to metolachlor were tested (Figure 1). In the absence of herbicide, there was very little leakage of ^{32}P from the roots of any of the species, indicating that the antibiotic and alcohol in the nutrient solutions had little if any effect on root permeability. Neither herbicide treated *Zea* nor *Pisum* (both metolachlor tolerant species) showed any significant leakage of ^{32}P from the roots compared to untreated plants, even at an herbicide concentration of 40 ppm. The two moderately susceptible species (tomato and wheat) showed three and four times the untreated plant level of ^{32}P leakage respectively, after 144 hours treatment with 30 ppm metolachlor. In the presence of 40 ppm metolachlor, however, ^{32}P leakage from these two species was 14 and 11 times the control rates respectively.

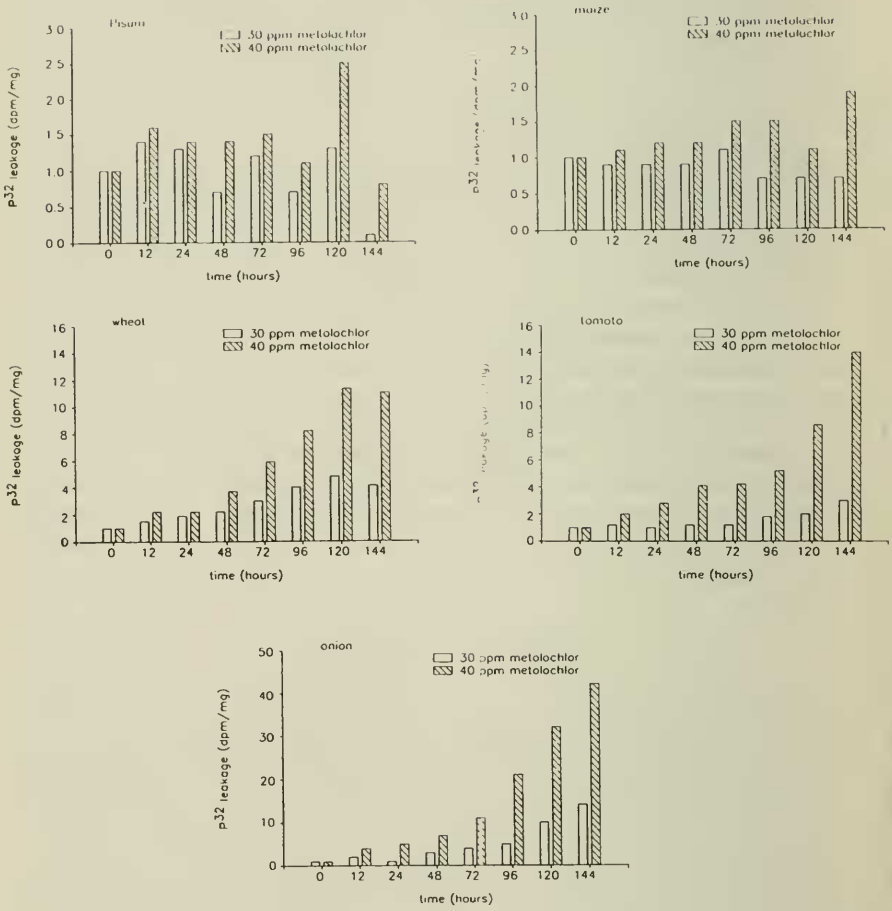

Figure 1. The effect of metolachlor on the leakage of previously absorbed ^{32}P from roots of five plants.

The maximum leakage of previously absorbed ^{32}P was from the roots of onion, a species highly sensitive to metolachlor. At 30 ppm metolachlor, ^{32}P leakage from onion roots was 14 times that of untreated roots after 144 hours. At 40 ppm metolachlor, ^{32}P leakage was 41 times the control value. Leakage of ^{32}P by onion roots was significantly different from the control after 72 hours with the 40 ppm metolachlor concentration. At its most extreme, the amount of ^{32}P released from herbicide treated onion roots was about 10% of the total amount absorbed. Because ^{32}P absorbed by roots is rapidly translocated to the shoot system, however, the amount lost would represent considerably more than 10% of the ^{32}P remaining in the roots. The reason for the differing rates of ^{32}P leakage, however, is not known. From these studies, it was not possible to determine whether metolachlor was exerting a direct effect on root membranes, or whether the leakage of exudates was a secondary effect resulting from an inhibition by the herbicide of some metabolic process related to membrane function or to the maintenance of membrane integrity.

Seed protectants can confer on germinating seeds a higher tolerance to herbicides and thus widen the margins of safety and selectivity. The potential of using naphthalic anhydride as a safener against metolachlor and alachlor injury has been tested, and it has been shown that naphthalic anhydride can prevent herbicide injury from these herbicides in sorghum (Ahrens & Davis 1978; Jordan & Jolliffe 1971 and Truelove & Davis 1977) and against alachlor injury to *Zea* (Burnside et al. 1971). These studies were conducted to determine whether naphthalic anhydride could protect onion roots from the permeability changes induced by metolachlor. The results are shown in Figure 2.

Metolachlor at a concentration of 30 ppm applied to onion roots via nutrient solution caused a 15 fold increase in the leakage of ^{32}P after 144 hours of treatment (Figure 2). When naphthalic anhydride at either 10 or 20 ppm was applied to onion roots with metolachlor at a concentration 30 ppm, however, very little ^{32}P leakage occurred. Thus, naphthalic anhydride protected the onion roots against permeability changes induced by metolachlor. Although the mechanism of action of metolachlor is unknown, the studies indicate that leakage of plant nutrients and loss of root cell membrane integrity are probably important factors in the mode of action of this herbicide.

Naphthalic anhydride applied alone at concentrations of 10 ppm did not cause leakage of ^{32}P from the roots. However, at high rates of application, naphthalic anhydride caused leakage of previously absorbed ^{32}P from onion roots. Such high rates of naphthalic anhydride did not protect onion roots from leakage induced by metolachlor. In fact, they promoted additional leakage.

St. John & Hilton (1973), reported the efflux of electrolytes from roots of wheat seedlings treated with 1×10^{-1}M dinoseb. They found that herbi-

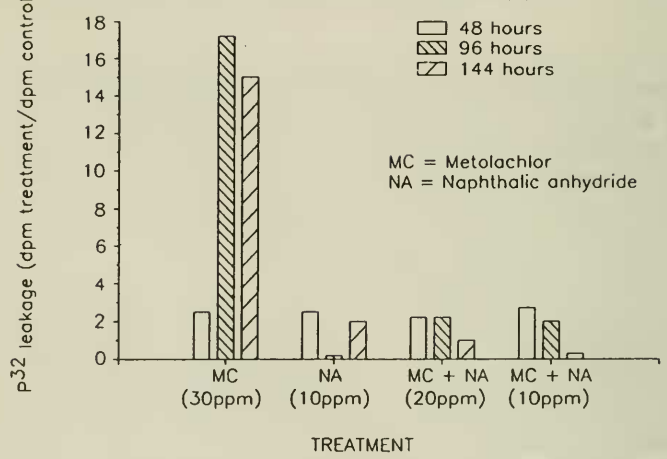

Figure 2. The effect of 1,8-naphthalic anhydride on metolachlor induced ^{32}P leakage from the roots of onion.

cide treatment reduced polar lipid levels by 72% and suggested that dinoseb may act by decreasing the levels of polar lipids required for membrane formation, hence altering membrane structure and function. Alteration of root membrane permeability characteristics, as shown by the leakage of plant exudates, suggested to us that metolachlor might also be acting through some effect on those metabolic processes involving membrane lipids. The effects of metolachlor and alachlor on lipid synthesis were determined by following the uptake and incorporation of radio labeled precursors into the lipids of excised wheat root tips. The uptake of the precursor, acetate-2-^{14}C, by both treated and untreated root tips was much greater than that of malonic acid-2-^{14}C (Figure 3). Between 56 and 63% of the applied acetate-2-^{14}C was absorbed by excised root tips, but only 7 to 9% of the applied malonic acid-2-^{14}C was absorbed. Neither alachlor nor metolachlor, however, affected the uptake of either of these precursors.

The radioactivity of the total lipid fraction extracted from herbicide treated tissue was not significantly different from that of untreated tissue, irrespective of which ^{14}C-labeled precursor was used in the study (Figure 3). The amount of radioactivity incorporated into the lipids, however, differed with the precursor used, with 7 to 9% of the applied acetate-2-^{14}C and 0.6 to 0.8% of the applied malonic acid-2-^{14}C incorporated into the lipids.

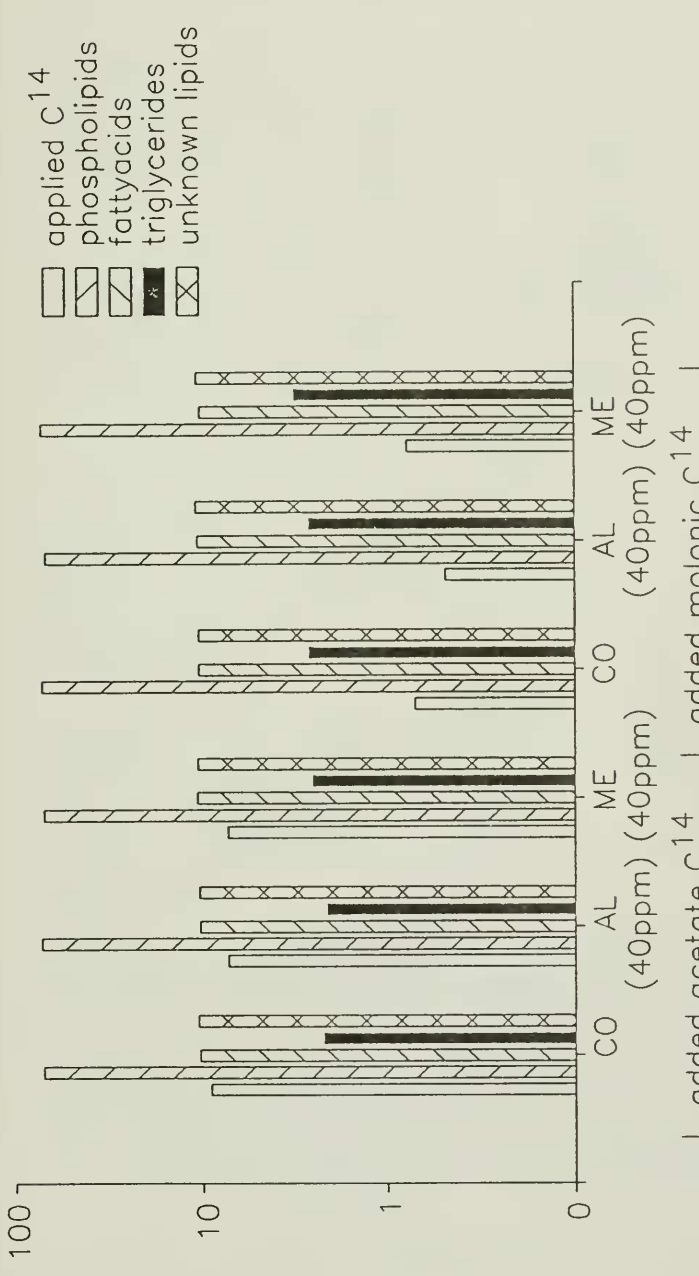

Figure 3. The effects of metolachlor on the incorporation of acetate-2-^{14}C and malonic acid-2-^{14}C into lipids of wheat root tips.

After separation of the total lipid extracts into the constituent lipid classes by TLC, the effect of the herbicide treatment on lipid synthesis could be determined. The incorporation of radio label into the different lipid classes was not inhibited by herbicide treatment. There were no significant differences in either the radio labeled phospholipid fraction or the neutral lipid classes in the total lipid extract.

The phospholipids were eluted from TLC plates and separated into the constituent phospholipid classes by TLC to determine whether alachlor or metolachlor inhibited the synthesis of specific phospholipids. Radio assay indicated that the synthesis of specific phospholipids was not inhibited by either herbicide.

No evidence was found to support the contention that a six hour exposure to alachlor or metolachlor inhibited lipid or phospholipid synthesis in excised wheat root tips.

The results of the investigation using a nine hour incubation period were similar to those for the six hour incubation, with no evidence of inhibition of either lipid or phospholipid synthesis by alachlor or metolachlor.

Earlier work had shown significant inhibition of total lipid synthesis by cotton root tips with a metolachlor treatment, although not with an alachlor treatment. More specifically, we had shown that total phospholipid synthesis was inhibited by both metolachlor and alachlor. Wilkinson (1981), failed to confirm these observations and there was no inhibition of lipid or phospholipid by either herbicide in this investigation.

To pursue these investigations further, wheat root tips were incubated with choline chloride-1,2-^{14}C, a precursor for phosphatidylcholine. After separation of the total lipid extract into the constituent lipid classes by TLC, the plates were radio scanned. The scans showed the presence of only two areas of radioactivity on the TLC plates, an area at the origin which accounted for 30% of the total radioactivity, and an area that corresponded to the phospholipid standard, phosphatidylcholine. The radioactivity at the origin was eluted from the silica gel and later identified as choline chloride-1,2^{14}C by paper chromatography. The results presented in Figure 4 show that the incorporation of choline chloride-1,2-^{14}C into phosphatidylcholine was not inhibited by metolachlor.

Thus, although metolachlor induces leakage of ^{32}P from the roots of susceptible species and causes subsequent loss of root cell membrane integrity, we have found no evidence, under these experimental conditions, that this loss of membrane integrity is due to the inhibition of total lipid, phospholipid or phosphatidylcholine synthesis by either alachlor or metolachlor.

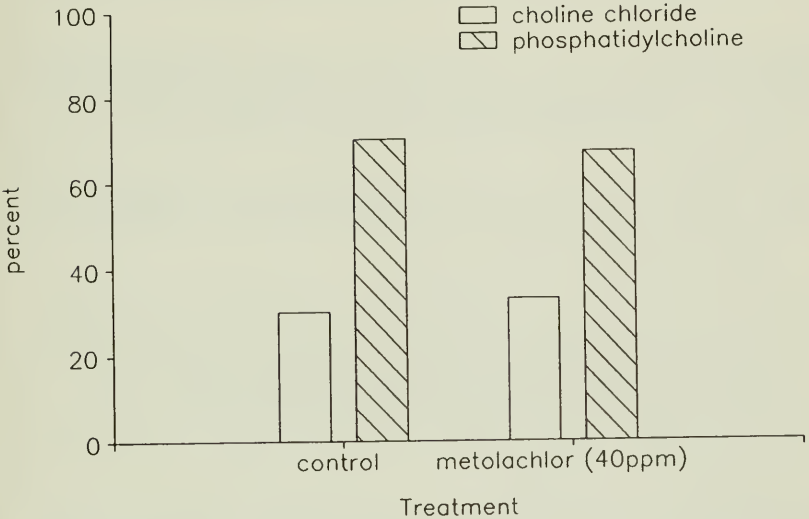

Figure 4. The effect of metolachlor on the incorporation of choline chloride 1,2-^{14}C into phosphatidylcholine.

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